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Test report

Determination of the disinfectant effect of the **Sani Table Plus** with test germ of Enterococcus faecium using the quantitative suspension test with germs according to the specifications of the standard test procedures of the German Society for Hygiene and Microbiology (DGHM)

Cooperation project between environmental hygiene Marburg GmbH & Co. KG and

Dr. Schmelz GmbH Malsfeld

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1. Question and description of the methodology:

The device Sani Table Plus generates a conversion of the oxygen in the air to hydroxide radicals by silent discharge at appropriate electrodes. The hydroxyl radicals represent the active agent.

For this purpose, the ambient air at the bottom of the device is sucked in by a fan and guided along the electrode surfaces in the device. The gas mixture air is conductive in the area of the electrode surfaces so that there is an atmospheric low-temperature plasma in the plasma state of the gas mixture air, the conversion of atmospheric oxygen to hydroxyl radicals described above is carried out.

Using a rotary potentiometer on the back, the electrode voltage can be continuously increased to 3500 volts (3.5 kV), so that ozone formation can also be used between 1750 volts and 3500 volts. This means that the device can optionally (!) also form ozone in addition to hydroxyl radicals, which could be used for forced disinfection or deodorization measures (removal of unpleasant odors).

The hydroxyl radicals are to be described as an active agent for the intended use. These have a strong denaturing (changing) property towards singular cells (bacteria, fungi) and on singular cell-like associations (viruses). Primarily unsaturated fatty acids in the fats of the cell membrane are oxidized. This destroys the cell membrane or increases the permeability. Cellular assemblies of higher cells, e.g. plant cells or animal cells, on the other hand, are not affected in any way, since the composition of the fats of the cell membranes is different.

With regard to silent electrical discharges with the formation of a plasma state, the formation of nitrogen oxides (NOx) is always possible. However, due to the chemical stability of the nitrogen gas molecule through a triple bond and the hybridization of the atoms involved, this is only possible if the electrode voltage is significantly above 3 kV. The tested device includes a technical limitation of the electrode voltage to 3.5 kV, so that nitrogen oxide formation is chemically and physically not possible. Therefore, the method can be called a safe method and is interesting with regard to the inactivation of microorganisms in continuous operation in the presence of people. The use of plasma, or the change in atmospheric oxygen into the plasma state, is already being used in certain technical niche areas: This includes the sterilization of returnable bottles before reuse, as well as the sterilization of refrigeration devices. The use of this method for wound disinfection is also being examined.

The plasma generator tested here has an air flow of 12mh. The use is recommended for rooms up to approx. 40m³, as well as in the near field of people who are to be protected against aerosol contamination. The plasma generator is also available in two higher power levels.

The potency of the inactivation of germs should be tested in a model test with the Enterococcus faecium test germ, which is a standard test germ for disinfection processes (e.g. dishwashers, washing machines, cleaning and disinfection devices).

If there is a corresponding inactivation performance against Enterococcus faecium, all covered (lipophilic) viruses are also recorded. Since certain viruses, e.g. viruses that trigger respiratory infections (e.g. rhinoviruses, coronaviruses) are transmitted aerogenically, use with appropriate antimicrobial efficacy results in the context of indoor air disinfection or continuous disinfection in the near field of people (e.g. in dental treatment or in public transport). At the same time, it is known that aerogenically emitted pathogens sediment on surfaces after an indefinite period. Disinfection by the method to be tested is also acceptable in the area of the surfaces.

The antimicrobial effectiveness is checked for this assessment by means of an inactivation test on surfaces. If inactivation can be recognized on the surfaces (as materially dense correlates), this also results in the fact that the air itself is also disinfected. This is possible because corresponding microorganisms are distributed in the air in a much lower number density than surfaces (gas versus

solid material of the surface). The test is carried out in a quantitative suspension test according to DGHM guidelines.

For this purpose, a test germ is applied to stainless steel sheets, then the test specimens are exposed to the method, the germs are then washed off and a decadal dilution series (in powers of ten) is applied. An aliquot of each dilution step is applied to a nutrient medium and then incubated. In parallel, a control sample without exposure is established to determine the initial number of bacteria (initial number of bacteria). The number of bacteria after exposure to the method is subtracted from the initial number of bacteria, thereby calculating the logarithmic reduction factor. This indicates by how many times an initial bacterial count is reduced by the procedure under consideration.

As a basic requirement of the DGHM for sufficient disinfection, a reduction performance of at least 3 powers of ten is required.

Since the method is only effective if the associated plasma generator is active, inactivation (disinfection of the disinfectant) is not necessary here. The disinfection time is limited solely by the exposure time of the test specimens compared to the procedure.

As a microbiocidal process, disinfection is intended to put an object, a surface or the air in a state of which there is no longer any risk of infection going out. This means that an initial germ load must be reduced to such an extent that the remaining germs no longer pose a risk of infection. As a rule, low residual background bacterial loads (e.g. 10 to 100 microorganisms per item) are unproblematic because the number of germs present is then usually below the number of infections. In this case, there is an aseptic condition that describes nothing other than the fact that there is no longer any risk of infection.

2. Material and methods:

2.1 Material

• Basic equipment of a microbiological laboratory:

pipettes, sterile

tweezers, sterile

vaccinations

Drigalski spatulas, sterile [1]

test tubes for dilution series, sterile

test tube racks o homogenizer (e.g. Vortex)

laboratory burner o incubator 36 ° C

Magnifying glass workplace with lighting for evaluation

Sterilizer

• Caso agar nutrient medium in petri dishes

• Columbia voli blood nutrient medium in petri dishes

• Saline solution 0.9% sterile

• Test germ Enterococcus faecium ATCC 6057 cultivated on nutrient medium

2.2 Method:

First, a pure culture of the test germ Enterococcus faecium ATCC 6057 is created on Columbia whole blood medium using a three-eye smear. Individual colonies are removed from the pure culture and spread out flat on another nutrient medium.

After incubation for 48 hours, the test germ has grown sufficiently and can be used to generate the test contamination.

For this purpose, the culture medium overgrown with the pure culture is loaded with 1.0 ml of saline solution, and the colonies are then washed away and suspended with a swab by rubbing the surface of the nutrient medium.

The germ suspension is then spread onto the test specimen using the swab. Sterile stainless steel sheets approx. 80 x 5 mm are used as test specimens. The stainless steel surface has a roughness depth of 100 µm.

Standard test specimen for the validation of the disinfection performance of cleaning and disinfection machines in the clinical environment.

After drying, the germs adhere to the test specimen. This can now be used for the examination.

To carry out the test, the test specimens are set up on a smooth, flat surface approx. 25 cm in front of the outlet opening of the plasma generator. The test specimens are slightly raised with a Drigalski spatula so that all surfaces (front and back) are in contact with the surrounding air.

Three different operating modes are tested:

• Test series 1 and 2 (V1 and V2):

o exposure time 30min, only plasma at 30% rel.

Moisture (half electrode voltage 1.75KV)

• Test series 3 and 4 (V3 and V4):

o exposure time 30min, only plasma at 95% rel.

Moisture (increased formation of hydroxyl ions) (half electrode voltage 1.75 kV)

• Test series 5 and 6 (V5 and V6):

o Exposure time 30 min, plasma and ozone in parallel (maximum electrode voltage 3.5 kV is set).

For each test, two test specimens are exposed and examined in parallel with the method. If the results differ greatly, the test is repeated with a larger number of test specimens in order to achieve homogeneity of the results by increasing the sample size.

The test is carried out in a room of the following dimensions:

• Length = 5.20 m, width = 2.57 m, height = 2.34 m

• Total volume = 31.27 m^3

• V1 and V2: the rel. Humidity of 30% is set by the HVAC and was available on the day of the test. Electrode voltage in the device approx. 1.75 kV

• V3 and V4: a petri dish with water is placed under the plasma generator so that the plasma generator sucks in air that is almost saturated with water vapor. Electrode voltage in the device approx. 1.75 kV

• V5 and V6: analogous to V1 and V2 (dry room air) without additional saturation. However, electrode voltage approx. 3.5 kV, so that ozone formation is possible.

To carry out the test, the ventilation and air conditioning system in the test room is switched off or deactivated.

After carrying out the exposure of the test specimens to the after inactivation method, the test specimens are again placed in sterile test tubes using sterile tweezers and kept available for the analysis to proceed.

A special inactivation of the disinfectant effect (so-called "disinhibition"), as initially noted, is not necessary since the method tested has no remanence, ie a lasting effect. The disinfectant effect is present as long as the method is active more lasting effect.

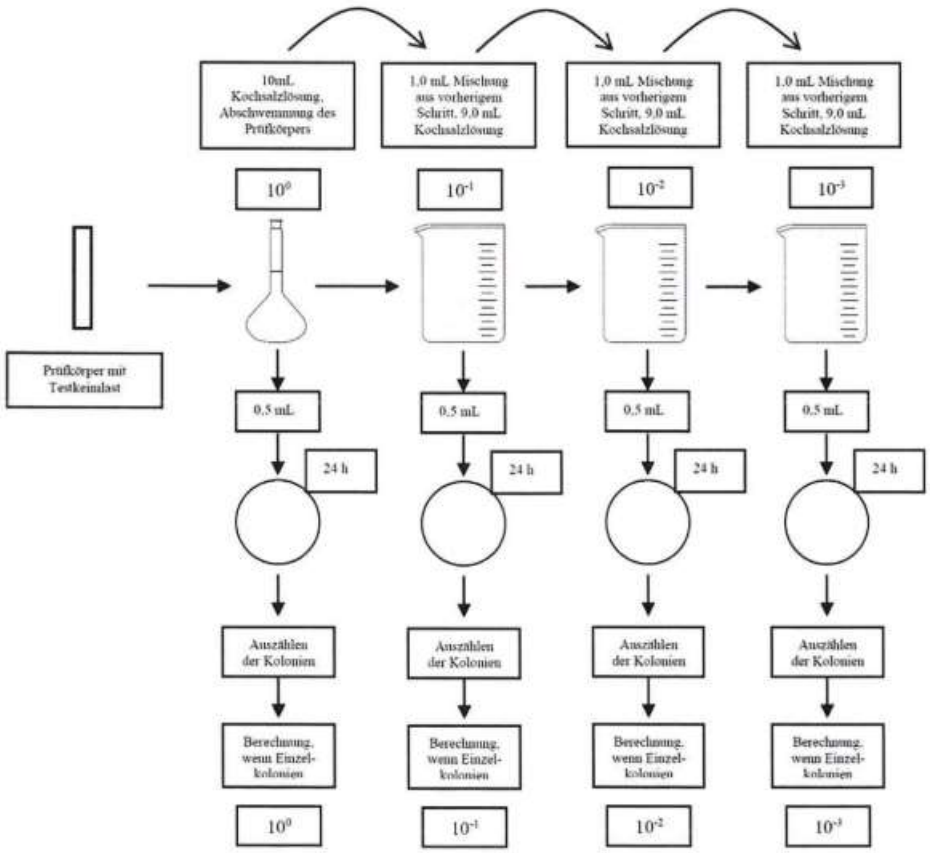
The test specimens are then transferred to a tube with 10 ml sterile saline and then shaken with the homogenizer. In this way, microorganisms adhering to the test specimen are transferred into the liquid phase. From this suspension, a dilution series in powers of ten up to 10-3 is created.

For this purpose, 1 mL of the suspension is pipetted onto the tube with the washed-off test specimen to 9 ml of saline in a new tube. This is homogenized, then 1 ml is taken from it again and the dilution series is continued accordingly.

Subsequently, a volume of 0.5mL is applied to each Caso-Agar medium from each dilution stage and then homogeneously distributed using a Drigalski spatula.

The culture media are then incubated in an incubator at 36 ° C for 24 hours.

After this time, the colonies are counted using the transmitted light method at 8 × magnification.



The nutrient medium of the dilution level, which has 10 to 200 colonies, is counted for the evaluation. Then the determined colony number is offset against the volume applied to the nutrient medium (0.5mL), as well as the initial volume of the dilution level (10mL) and the respectively counted dilution level.

The number of bacteria per test specimen is thereby calculated. The decimal logarithm is then calculated.

The logarithm of the bacterial count of the test specimen exposed to the inactivation method is subtracted from the logarithm of the bacterial count of the test specimen without exposure (control sample).

In this way, the logarithmic reduction factor (LRF) is obtained. The LRF specifies by how many powers of ten the initial number of bacteria is reduced.

According to the recommendations of the DGHM, sufficient disinfection should reduce the bacterial count by 3 powers of ten (= 1000-fold bacterial count reduction).

Then 99.9% of the existing microorganisms are killed, so that the disinfected surfaces or areas are usually no longer infectious.

4. Interpretation, evaluation and recommendations:

The results show a jog with the occurrence of hydroxy radicals (oxygen plasma) in dry air (approx. 30% relative humidity) for 30 min, reduction factor of 5.38.

Increased formation of hydroxyl radicals under water vapor saturation becomes a little higher over 30 min. Reduction factor of 6.04 is achieved.

A reduction factor of 8.04 is achieved even with parallel activation of the ozone synthesis over 30 minutes.

Sufficient disinfection exists if the number of germs is reduced by at least 3 powers of ten, i.e. a reduction factor of 3 1000 times. That corresponds to a log. A reduction factor of 5.38 corresponds to 109 = 239,883 (> 5 powers of ten).

This means that out of 239,883 germs one germ is still viable. Of 6.04 corresponds to 106.0 = 1,096,478 (> 6 a reduction factor of powers of ten). This means that out of 1,098,478 germs, one germ is still viable. This means that the requirements for adequate disinfection under the tested conditions have been fully met in the quantitative suspension experiment.

This applies both under normal ambient conditions without ozone synthesis (experiments 01 and 02, LRF 5.38), water vapor saturation (experiments 03 and 04, LRF 6.04) and under additional ozone synthesis (experiments 05 and 06, LRF 8.04). Also maximum hydroxyl radical formation at as below the difference of an LRF between 6.04 and 5.38 is insignificant with regard to disinfection, therefore a sufficient disinfection effect is achieved under all three tested operating modes / operating conditions.

The optional activatable parallel ozone synthesis can be used as additional security or for the purpose of deodorization (odor removal) from rooms. An identical test was carried out in a preliminary test. The difference was in the organic burden.

The test in the preliminary test was carried out under high organic load (3% shear erythrocytes in the germ suspension). The preliminary test showed no significant reduction in the number of bacteria, which means that the number of bacteria was almost unchanged even when exposed to BE compared to the control sample.

The results of a sufficient germ count reduction in the current study were determined without additional organic load. This means that an organic load reduces the effect of the inactivation process, or Inactivation process based on hydroxyl radicals works and only removes them in the. This observation is understandable because the Gas phase (air) and on surfaces are effective, but not within contaminations. Germs trapped in contaminants are therefore not inactivated.

This leads to the following conclusions:

The method shows a clear microbicidal effectiveness in the near field of the plasma generator with little or absent organic contamination.

The effectiveness corresponds to> 5 or> 6 powers of ten to reduce the number of germs to disinfection in the sense of asepsis, i.e. the reduction in the number of bacteria leads to a condition in the treated area which is no longer infectious. A potentially infectious initial bacterial count is reduced to such an extent that there is no longer any risk of infection.

The disinfection effectiveness was confirmed on the test germ Enterococcus faecium. This test germ is established as a model organism for the testing of disinfection processes in a clinical environment (dishwashers, cleaning and disinfection devices (RDGS), bedpan washers, industrial washing machines, etc.).

Destruction of the Enterococcus faecium test germ shows that the disinfection procedure is effective in the following classes of action according to the RKI list:

Class A: complete • Action against native bacteria with the exception of bacterial endospores, as well as against fungi.

Class B: limited, only effective against enveloped (lipophilic) viruses. The inactivating effect against enveloped viruses is identical to the effect in activity class A, with regard to viruses the effect is only given against enveloped viruses, but not against non-enveloped viruses (eg norovirus, rotavirus, hepatits A virus).

This means that the inactivation test against Enterococcus faecium is representative or equivalent to the inactivation of enveloped viruses.

However, the effectiveness within contaminations is not given. The process is effective on the surface and in the air.

Enveloped viruses have plasma membranes derived from the host cell. This is due to the fact that such viruses are released from the host cell by controlled exocytosis (ejection). During the discharge, the plasma membrane wraps itself around the virus particle and forms the virus envelope. The virus envelope conveys the infectivity of enveloped viruses. Because the phospholipids of the virus envelope are already changed and destroyed by atmospheric oxygen and dehydration, enveloped viruses usually lose their infectivity after 3 to 5 days in the ambient air (e.g. on surfaces). However, this time interval is too long. In the meantime, possible contaminations from corresponding chains of infection and thus infectious hazards can arise. Enveloped viruses are usually transmitted hematogenously or aerogenically and rarely via contact contamination.

By means of a shell made of phospholipids which, by comparison, uncovered viruses are released by destroying the host cell that is to say in an uncontrolled manner.

As a result, the phospholipid envelope around the virus particles is missing. Such virus particles consist of resistant protein compounds (capsids) that only can be destroyed by more potent substances (e.g. halogen compounds, such as chlorine or chlorine dioxide and peroxides, as well as aldehydes). Therefore, non-enveloped viruses are very resistant to the environment and inactivation. Most of the time, non-enveloped viruses are transmitted orally or via contact contamination.

The tested disinfection process reduces the time interval required for the natural destruction of enveloped viruses from 3 to 5 days to approx. 30 minutes, or can be operated continuously, which leads to a continuous disinfection of the air and the surfaces in the near field.

Evidence of a surface disinfection effect in the vicinity of the device also indicates air disinfection, since the disinfection agent (hydroxyl radicals) is contained in the air discharged from the device. When the effect occurs at the target location (here: on the test specimens in the vicinity of the device), the air emitted from the plasma generator is also disinfected.

Since the tested Enterococcus faecium shows and thus the active classes A and limited B (only enveloped viruses) are recorded, the use of the method is particularly interesting against certain aerogenically transmitted viruses. Disinfection procedures and effectiveness against this include certain rhinoviruses, influenza viruses and especially corona viruses.

This also includes the corona virus "SARS-CoV-2", which was first described in December 2019.

There are therefore interesting possible uses of the tested disinfection process in areas in which the distance of 1.5 to 2 m required to interrupt the transmission of aerogenic infections cannot be maintained.

From the point of view of microbiology and epidemiology, the following areas of application are particularly recommended:

- Patient-touching situations in medical care and elderly care (patient rooms, diagnostic rooms, treatment rooms, living rooms / lounges in old people's homes, medical supply stores, etc.)

- Dental care: this is precisely where treatment is concerned Aerosols are released, which remain in the air and which are occasionally exposed to subsequent patients and to which dental personnel are permanently exposed.

- Sale of goods and item registration / cash register: here, too, the risk of infection can be reduced by additional air / surface disinfection in the vicinity of the employees

- Furthermore, the use in ventilation and air conditioning systems is recommended.

These examples show various other possible applications.

Taken as a whole, the method represents a proven means of reducing the bacterial count of aerogenically carried microorganisms and microorganisms sedimented on surfaces in the vicinity of the device. The activity is effective against native bacteria and fungi, as well as enveloped viruses (including the SARS-CoV-2 virus).

The procedure cannot and should not replace surface disinfection. It is also not intended to be a sole method of risk reduction and also to replace further hygiene measures for risk reduction.

It is important that the method tested is a very effective addition to the prevention of transmission of aerogenic agents. In the case of aerogenic transmissions, the relevant transmission path (via the air) is prevented.

In the "hurdle concept" of prevention, several hurdles are put in the way of a possible transmission of a microorganism. The more hurdles are built up, the more effective the prevention. And in this context, the tested method represents a valuable and important hurdle for microorganisms, because here especially the medium of transmission, ie the air, is disinfected and possible surface sedimentation from aerosols is eliminated.